

Claims

1. An osteogenic device for implantation in a mammal, said device comprising:

a biocompatible, in vivo biodegradable matrix defining pores of a dimension sufficient to permit influx, proliferation and differentiation of migratory progenitor cells from the body of said mammal; and

a protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains, each of which has an amino acid sequence sufficiently duplicative of the sequence of COP-5 or COP-7 such that said protein is capable of inducing endochondral bone formation in association with said matrix when implanted in a mammal.

2. A device for implantation in a mammal, said device comprising:

a biocompatible, in vivo biodegradable matrix defining pores of a dimension sufficient to permit influx, proliferation and differentiation of migratory progenitor cells from the body of said mammal; and

a protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains, each of which has less than about 200 amino acids, in a sequence sufficiently duplicative of the sequence of

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COP-5 or COP-7 such that said protein is capable of inducing cartilage formation in association with said matrix when implanted in a mammal.

3. The device of claim 1 or 2 wherein the sequence comprises:

10 20 30 40 50
CXXXXLXVXFDXGWWXXXPXXGXXAXYCXGX~~XXX~~PXXXXXXNHAXX
60 70 80 90 100
QXXVXXXXNXXXXPXXCCXPXXXXXXLXXXXXXVXLXXX~~XXX~~MXVXXCXCX

wherein each X independently represents an amino acid.

4. The device of claim 1 or 2 wherein the sequence comprises:

10 20 30 40 50
LXVXFDXGWWXXXPXXGXXAXYCXGX~~XXX~~PXXXXXXNHAXX
60 70 80 90 100
QXXVXXXXNXXXXPXXCCXPXXXXXXLXXXXXXVXLXXX~~XXX~~MXVXXCXCX

wherein each X independently represents an amino acid.

5. The device of claim 1 or 2 wherein the sequence comprises:

10 20 30 40 50
CKRHPLYVDFRDVGWNDWIVAPP~~GY~~HAFYCHGECPFPLADHLNSTNHAIV
RRRS K S S L QE VIS E FD Y E A AY MPESMKAS VI
KE F E K I DN L N S Q ITK F P TL
Q A S K
60 70 80 90 100
QTLVNSVNP~~G~~KIPKACCVPTELSAISMLYLDENENVVLKNYQDMVVEGCGCR
SI HAI SEQV EP A EQMNSLAI FFNDQDK I RK EE T DA H H
RF T S K DPV V Y N S H RN RS
N S K P E

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

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6. The device of claim 1 or 2 wherein the sequence comprises:

10 20 30 40 50
LYVDFRDVGWNDWIVAPPGYHAFYCHGECPFPLADHLNSTNHAIV
K S S L QE VIS E FD Y E A AY MPESMKAS VI
F E K I DN L N S Q ITK F P TL
A S K
60 70 80 90 100
QTLVNSVNPKGKIPKACCPTELSAISMLYLDENENVVLKNYQDMVVEGCGCR
SI HAI SEQV EP A EQMNSLAI FFNDQDK I RK EE T DA H H
RF T S K DPV V Y N S H RN RS
N S K P E

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

7. The device of claim 1 or 2 wherein the sequence comprises:

Vgl 1 10 20 30 40
CKKRHLVYEFK-DVGWQNWVIAPIQGYMANCYGECPYPLTE
50 60 70
ILNGSN--H-AIQLTLVHSIEPED-IPLPCCVPTKMSPI
80 90 100
ISMLFYDNNNDNVVLRHYENMAVDECGR

8. The device of claim 1 or 2 wherein the sequence comprises:

DPP 1 10 20 30 40
CRRHSLYVDFS-DVGWDDWIVAPLGYDAYYCHGKCPFPLAD
50 60 70
HFNSTN--H-AVVQTLVNNNNPGK-VPKACCPVPTQLDS
80 90 100
VAMLYLNDQSTVVLKNYQEMTVVCGCR

9. The device of claim 1 or 2 wherein the sequence comprises:

OP1 1 10 20 30 40
LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
50 60 70
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
80 90 100
ISVLYFDDSSNVILKKYRNMVVRACGCH

10. The device of claim 1 or 2 wherein the sequence comprises:

OP1 1 10 20 30 40
CKKHELYVSFR-DLGWQDWI LAPEGYAAYYCEGECAFPLNS
50 60 70
YMNATN--H-AIVQTLVHF INPET-VPKPCCAPTQLNA
80 90 100
ISVLYFDDSSNVILKKYRNRMVVRACGCH

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11. The device of claim 1 or 2 wherein the sequence comprises:

CBMP-2a 1 10 20 30 40
CKRHPLYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD
50 60 70
HLNSTN--H-AIVQTLVNSVNS-K-IPKACCVPTELSA
80 90 100
ISMLYLDENEKVVVLKNYQDMVVEGCGCR

12. The device of claim 1 or 2 wherein the sequence comprises:

CBMP-2b 1 10 20 30 40
CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGDCPFPLAD
50 60 70
HLNSTN--H-AIVQTLVNSVNS-S-IPKACCVPTELSA
80 90 100
ISMLYLDEYDKVVLKNYQEMVVEGCGCR

13. The device of claim 1 or 2 wherein the sequence comprises:

CBMP-3 1 10 20 30 40
CARRYLKVDFA-DIGWSEWIISPKSFDAYYCSGACQFPMPK
50 60 70
SLKPSN--H-ATIQSIVRAVGVVPGIPEPCCVPEKMSS
80 90 100
LSILFFDENKNVVLKVYPNMTVESACR

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14. The device of claim 1 or 2 wherein the sequence comprises:

COP1 1 10 20 30 40
LYVDFQRDVGVDDWIIAPVDFDAYYCSGACQFPSAD
50 60 70
HFNSTN--H-AVVQTLVNNMNPKG-VPKPCCVPTELSA
80 90 100
ISMLYLDENSTVVLKNYQEMTVVCGCGR

15. The device of claim 1 or 2 wherein the sequence comprises:

COP3 1 10 20 30 40
LYVDFQRDVGVDDWIVAPPGYQAFYCSGACQFPSAD
50 60 70
HFNSTN--H-AVVQTLVNNMNPKG-VPKPCCVPTELSA
80 90 100
ISMLYLDENEKVVVLKNYQEMVVEGCGCR

16. The device of claim 1 or 2 wherein the sequence comprises:

COP4 1 10 20 30 40
LYVDFS-DVGWDDWIVAPPGYQAFYCSGACQFPSAD
50 60 70
HFNSTN--H-AVVQTLVNNMNPKG-VPKPCCVPTELSA
80 90 100
ISMLYLDENEKVVVLKNYQEMVVEGCGCR

17. The device of claim 1 or 2 wherein the sequence comprises:

COP5 1 10 20 30 40
LYVDFS-DVGWDDWIVAPPGYQAFYCHGECPFPLAD
50 60 70
HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
80 90 100
ISMLYLDENEKVVVLKNYQEMVVEGCGCR

18. The device of claim 1 or 2 wherein the sequence comprises:

COP7 1 10 20 30 40
LYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD
50 60 70
HLNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
80 90 100
ISMLYLDENEKVVVLKNYQEMVVEGCGCR

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19. The device of claim 1 or 2 wherein the sequence comprises:

COP16

1 10 20 30 40
CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGECPFPLAD
50 60 70
HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
80 90 100
ISMLYLDENEKVVVLKNYQEMVVEGGCR

20. The device of claim 1 or 2 wherein the osteogenics protein comprises a pair of separate polypeptide chains.

21. Osteogenic protein, produced by expression of recombinant DNA in a host cell, capable of inducing endochondral bone formation in association with a matrix when implanted in a mammal.

22. A protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains less than about 200 amino acids long in a sequence sufficiently duplicative of the sequence of COP-5 or COP-7 such that said protein is capable of inducing cartilage formation in association with a matrix when implanted in a mammal.

23. ² _a The osteogenic protein of claim ²¹ ₂₁ having an apparent molecular weight of about 30 kD when oxidized as determined by comparison to molecular weight standards in SDS-polyacrylamide gel.

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3 24. The osteogenic protein of claim 23
further characterized by being glycosylated.

4 25. The osteogenic protein of claim 21 having
an apparent molecular weight of about 27 kD as
determined by comparison to molecular weight
standards in SDS-polyacrylamide gel
electrophoresis.

5 26. The protein of claim 22 or 25 further
characterized by being unglycosylated.

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27. The protein of claim 21 or 22 comprising
a pair of separate polypeptide chains.

6 28. The protein of claim 21 or 22 comprising the
amino acid sequences:

T920X
10 20 30 40 50
CXXXXLXVXFDXGWWXXXPXXGXXAXYCXGXCXXPXXXXXXNHAXX
60 70 80 90 100
QXXVXXXXNXXXXPXXCCXPXXXXXXLXXXXXXVXLXXYXXMXVXXCXCX

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wherein each X independently represents an amino acid.

7 29. The protein of claim 21 or 22 comprising the
amino acid sequences:

T921X
10 20 30 40 50
LXVXFDXGWWXXXPXXGXXAXYCXGXCXXPXXXXXXNHAXX
60 70 80 90 100
QXXVXXXXNXXXXPXXCCXPXXXXXXLXXXXXXVXLXXYXXMXVXXCXCX
wherein each X independently represents an amino acid.

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30. The protein of claim 21 or 22 comprising the amino acid sequences:

10 20 30 40 50
CKRHPLYVDFRDVGWNDWIVAPPGYHAFYCHGECFPPLADHLNSTNHAIV
RRRS K S S L QE VIS E FD Y E A AY MPESMKAS VI
KE F E K I DN L N S Q ITK F P TL
Q A S K
60 70 80 90 100
QTLVNSVNPKGKIPKACCPTELSAISMLYLDENENVVLKNYQDMVVEGCGCR
SI HAI SEQV EP A EQMNSLAI FFNDQDK I RK EE T DA H H
RF T S K DPV V Y N S H RN RS
N S K P E

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

31. The protein of claim 21 or 22 comprising the amino acid sequences:

10 20 30 40 50
LYVDFRDVGWNDWIVAPPGYHAFYCHGECFPPLADHLNSTNHAIV
K S S L QE VIS E FD Y E A AY MPESMKAS VI
F E K I DN L N S Q ITK F P TL
A S K
60 70 80 90 100
QTLVNSVNPKGKIPKACCPTELSAISMLYLDENENVVLKNYQDMVVEGCGCR
SI HAI SEQV EP A EQMNSLAI FFNDQDK I RK EE T DA H H
RF T S K DPV V Y N S H RN RS
N S K P E

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

32. The protein of claim 21 or 22 comprising the amino acid sequences:

Vgl 1 10 20 30 40
CKKRHLYVEFK-DVGWQNWVIAPQGYMANYCYGECPYPLTE
50 60 70
ILNGSN--H-AILQTLVHSIEPED-IPLPCCVPTKMSP
80 90 100
ISMLFYDNNNDNVVLRHYENMAVDECGR

33. The protein of claim 21 or 22 comprising the amino acid sequences:

DPP 1 10 20 30 40
CRRHSLYVDFS-DVGWDDWIVAPLGYDAYYCHGKCPFPLAD
50 60 70
HFNSTN--H-AIVQTLVNNNNPGK-VPKACCVPTQLDS
80 90 100
VAMLYLNDQSTVVLKNYQEMTVVCGCGR

34. The protein of claim 21 or 22 comprising the amino acid sequence:

OP1 1 10 20 30 40
LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
50 60 70
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPQLNA
80 90 100
ISVLYFDDSSNVILKKYRNRMVVVRACGCH

35. The protein of claim 21 or 22 comprising the amino acid sequences:

OP1 1 10 20 30 40
CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
50 60 70
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPQLNA
80 90 100
ISVLYFDDSSNVILKKYRNRMVVVRACGCH

36. The protein of claim 21 or 22 comprising the amino acid sequences:

CMP-2a 1 10 20 30 40
CKRHPLYVDFS-DVGWDDWIVAPPGYHAFYCHGECPFPLAD
50 60 70
HLNSTN--H-AIVQTLVNSVNS-K-IPKACCVPTELSA
80 90 100
ISMLYLDENEKVVLKNYQDMVVECGCGR

37. The protein of claim 21 or 22 comprising the amino acid sequences:

CBMP-2b 1 10 20 30 40
CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGDCPFPLAD
50 60 70
HLNSTN--H-AIVQTLVNSVNS-S-IPKACCVPTELSA
80 90 100
ISMLYLDEYDKVVLKNYQEMVVEGCGCR

38. The protein of claim 21 or 22 comprising the amino acid sequences:

CBMP-3 1 10 20 30 40
CARRYLKVDFA-DIGWSEWIISPKSFDAYYCSGACQFPMPK
50 60 70
SLKPSN--H-ATIQSIVRAVGVVPGIPEPCCVPEKMSS
80 90 100
LSILFFDENKNVVLKVYPNMTVESACR

39. The protein of claim 21 or 22 comprising the amino acid sequences:

COP1 1 10 20 30 40
LYVDFQRDVGVDDWIIAPVDFDAYYCSGACQFPSAD
50 60 70
HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
80 90 100
ISMLYLDENSTVVLKNYQEMTVVCGCR

40. The protein of claim 21 or 22 comprising the amino acid sequences:

COP3 1 10 20 30 40
LYVDFQRDVGVDDWIVAPPGYQAFYCSGACQFPSAD
50 60 70
HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
80 90 100
ISMLYLDENEKVLKNYQEMVVEGCGCR

41. The protein of claim 21 or 22 comprising the amino acid sequences:

COP4 1 10 20 30 40
LYVDFS-DVGWDDWIVAPPGYQAFYCSGACQFPSAD
50 60 70
HFNSTN--H-AVVQTLVNNMNPGK-VPKPCCVPTELSA
80 90 100
ISMLYLDENEKVLKNYQEMVVEGCGCR

42. The protein of claim 21 or 22 comprising the amino acid sequences:

COP5 1 10 20 30 40
 LYVDFS-DVGWDDWIVAPPGYQAFYCHGECPFPLAD
 50 60 70
 HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
 80 90 100
 ISMLYLDENEKVVVLKNYQEMVVECGCGR

43. The protein of claim 21 or 22 comprising the amino acid sequences:

COP7 1 10 20 30 40
 LYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD
 50 60 70
 HLNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
 80 90 100
 ISMLYLDENEKVVVLKNYQEMVVECGCGR

44. The protein of claim 21 or 22 comprising the amino acid sequences:

COP16 1 10 20 30 40
 CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGECPFPLAD
 50 60 70
 HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
 80 90 100
 ISMLYLDENEKVVVLKNYQEMVVECGCGR

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45. The protein of claim 21 or 22 comprising the product of expression of a DNA in a prokaryotic cell.

46. A DNA sequence encoding an amino acid sequence sufficiently duplicative of that of the sequence encoded by the gene of Figure 1A such that said encoded sequence induces bone or cartilage formation when implanted in a mammal in association with a matrix.

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47. The DNA of claim 46 encoding the same amino acid sequence as the gene set forth in Figure 1A.

48. The DNA sequence of claim 46 encoding:

OP1 1 10 20 30 40
LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
50 60 70
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPQLNA
80 90 100
ISVLYFDDSSNVILKKYRNMVVVRACGCH

49. The DNA sequence of claim 46 encoding:

OP1 1 10 20 30 40
-5
HQRQA
CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS
50 60 70
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPQLNA
80 90 100
ISVLYFDDSSNVILKKYRNMVVVRACGCH

50. A cell line engineered to express the protein of claim 21 or 22.

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51. The protein of claim 21 having a half maximum bone forming activity of about 20 - 25 ng per 25 mg of implant.

52. A biocompatible, in vivo biodegradable deglycosylated collagenous matrix defining pores of dimensions sufficient to permit influx, proliferation, and differentiation of migratory progenitor cells from the body of a mammal.

53. The matrix of claim 52 comprising close-packed particulate matter having a particle size within the range of 70-850 μ m.

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54. The matrix of claim 53 wherein said particulate matter has a particle size within the range of 70-420 mm.

55. The matrix of claim 52 defining a shape to span a non-union fracture in said mammal.

56. The matrix of claim 52 comprising demineralized, protein-extracted, deglycosylated, particulate xenogenic bone.

57. The matrix of claim 52 comprising a material selected from the group consisting of hydroxyapatite, tricalcium phosphate, polymers comprising lactic acid monomer units, polymers comprising glycolic acid monomer units, demineralized, guanidine-extracted, deglycosylated xenogenic bone, and mixtures thereof.

58. An osteogenic device for implantation in a mammal, said device comprising:

a biocompatible, in vivo biodegradable matrix defining pores of a dimension sufficient to permit influx, proliferation and differentiation of migratory progenitor cells from the body of said mammal; and

substantially pure osteogenic protein capable of inducing endochondral bone formation in said mammal disposed in said matrix and accessible to said cells.

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59. The device of claim 1, 2, or 58 wherein said matrix comprises close-packed particulate matter having a particle size within the range of 70-850 mm.

60. The device of claim 1, 2, or 58 wherein said particulate matter has a particle size within the range of 70-420 mm.

61. The device of claim 1, 2, or 58 wherein said matrix comprises demineralized, protein-extracted, particulate, allogenic bone.

62. The device of claim 1, 2, or 58 wherein said matrix comprises a material selected from the group consisting of collagen, hydroxyapatite, tricalcium phosphate, polymers comprising lactic acid monomer units, polymers comprising glycolic acid monomer units, demineralized, guanidine-extracted allogenic bone, and mixtures thereof.

63. The device of claim 1, 2, or 58 wherein said matrix is shaped to span a non-union fracture in said mammal.

64. The device of claim 1, 2, or 58 disposed within the marrow cavity of allogenic bone.

65. The device of claim 1, 2, or 58 wherein said matrix comprises demineralized, protein extracted, particulate, deglycosylated xenogeneic bone.

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66. The device of claim 65 wherein said matrix is treated with a protease.

67. The device of claim 58 wherein said osteogenic protein is unglycosylated.

68. The device of claim 67 wherein said osteogenic protein has an apparent molecular weight of about 27 kD when oxidized as determined by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

69. The device of claim 58 wherein said osteogenic protein is glycosylated.

70. The device of claim 69 wherein said osteogenic protein has an apparent molecular weight of about 30 kD when oxidized as determined by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

71. The device of claim 58 wherein said osteogenic protein comprises a pair of polypeptide chains.

72. The device of claim 71 wherein one chain of said pair of polypeptide chains has an apparent molecular weight of about 14 kD and the other has an apparent molecular weight of about 16 kD, both as determined after reduction by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

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73. The device of claim 71 wherein one chain of said pair of polypeptide chains has an apparent molecular weight of about 16 kD and the other has an apparent molecular weight of about 18 kD, both as determined after reduction by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

74. The device of claim 58 wherein said osteogenic protein has the approximate amino acid composition set forth below:

<u>Amino acid residue</u>	<u>Rel. no. res./molec</u>	<u>Amino acid residue</u>	<u>Rel. no. res./molec.</u>
Aspartic acid/	22	Tyrosine	11
Asparagine		Valine	14
Glutamic acid/	24	Methionine	3
Glutamine		Cysteine	16
Serine	24	Isoleucine	15
Glycine	29	Leucine	15
Histidine	5	Proline	14
Arginine	13	Phenylalanine	7
Threonine	11	Tryptophan	ND
Alanine	18		
Lysine	12		

75. The device of claim 58 wherein said osteogenic protein comprises the amino acid sequence:

VPKPCCAPT

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76. The device of claim 1 or 58 wherein the half maximum bone inducing activity of said protein is 0.8 to 1.0 ng per mg of said matrix.

77. A method of inducing local cartilage or bone formation in a mammal comprising the step of implanting the device of claim 1, 2, or 58 in said mammal at a locus accessible to migratory progenitor cells of said mammal.

78. A method of inducing endochondral bone formation in a mammal comprising the step of implanting the device of claim 1 or 58 in said mammal at a locus accessible to migratory progenitor cells of said mammal.

79. A method of inducing endochondral bone formation in a non-union fracture in a mammal comprising the step of implanting in the fracture in said mammal the device of claim 63.

80. Antibodies reactive with an epitope of the protein of claim 21 or 22.

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H2
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